THE EFFECT OF ROENTGEN RAY AND THORIUM X ON PNEUMOCOCCUS AND STREPTOCOCCUS INFECTIONS IN MICE

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In many of the acute infectious diseases, e. g., typhoid fever, measles, and influenza, leukopenia is a characteristic blood change and of some diagnostic value. These diseases are frequently complicated by what is supposed to be a secondary or concurrent infection with pneumococci and streptococci so that the question has arisen whether the diseases themselves are not caused by organisms of the pneumococcus or streptococcus group. There may be two important sources of leukopenia, the first and probably more permanent and serious one being injury or destruction of the hematopoietic organs, such as can be produced experimentally by the use of benzene, the roentgen ray, and certain radioactive preparations, and the other which may only be a precursor to a leukocytosis may be the introduction of foreign or bacterial proteins [1]-intravenously. In view of the conception of the intimate relationship between the leukocyte and antibody formation in combating acute infections, and having found that even a profound injury or destruction of the hematopoietic organs by means of benzene, roentgen ray and thorium X had no appreciable effect on the progress of experimental tuberculosis,[2] it seemed desirable to study the effect of the roentgen ray and thorium X on pneumococcus and streptococcus infections.

Kellert [3] noted no effect of the roentgen ray on experimental tuberculosis in the guinea-pig, but his animals seemed to be more susceptible to spontaneous acute infections after being exposed to the ray. The effect of benzene, roentgen ray and thorium X on antibody formation has been studied by Hektoen,[4] who found that these preparations all produced a marked leukopenia with grave lesions in the marrow and that benzene in rabbits greatly reduced the production of specific precipitin and lysin for sheep blood; and that exposure of white rats, and dogs and rabbits to the roentgen ray restrains the formation of specific

Received for publication Aug. 16, 1920.

[1] Wells, Clifford W., Jour. Infect. Dis., 1917, 20, p. 219.
[2] Corper, H. J., Amer. Rev. Tuberc., 1918, 2, p. 587.
[3] Kellert, Ellis: Jour. Med. Res., 1918, 39, p. 93.
Jour. Infect. Dis., 1916, 19, p. 69; 1915, 17, p. 415; 1918, 22, p. 28.

lysin for sheep blood to a marked degree. Hektoen and Corper[5] found that thorium X might reduce specific precipitins even in the absence of a definite reduction in the leukocytes in the peripheral blood while lysins for sheep blood were little affected by it. Hektoen states that "benzene may lower the anti-infectious powers of the body in at least three ways: by the reduction of antibodies, by the reduction of the number of leukocytes, and by the reduction of the phagocytic activity of the leukocytes." This statement probably applies equally well to all three substances.

Läwen[6] found that prolonged roentgen-ray exposure of rabbits increases their susceptibility to staphylococcus infections. The resistance of mice to pyocyaneus and anthrax, and rats to anthrax infections was reduced by roentgenization. If rats and rabbits were first exposed not too long and then infected with anthrax and typhoid bacilli, respectively, there resulted a consequent increase of leukocytes as compared to animals only exposed to the roentgen rays. Roentgenization had no effect on the bactericidal action of normal serum of rabbits, guinea-pigs, and rats toward typhoid bacilli. Roentgen ray had no effect on the normal typhoid, colon and pyocyaneus agglutinins in rabbits; while the formation of specific typhoid agglutinins was distinctly depressed; it had no effect on specific typhoid agglutinins in vitro nor those contained in rabbit serum after passive transfer. There was also a marked retardation of the formation of specific typhoid bacteriolysins in roentgenized rabbits.

Regarding the effect of severe leukopenia on inflammatory reactions, Camp and Baumgarten[7] found that a congestion of blood vessels and marked edema may occur in rabbits with severe benzene leukopenia, these two phenomena being independent of the leukocytes. When the leukocyte count is below 1000, croton oil and heat produced no leukocytic exudate in the tissues of the ear, and carmine produced no exudate in the muscles. Benzene administration did not, however, destroy the leukocytes in an abscess.

The effect of leukopenia on experimental pneumococcus infections was studied especially by Winternitz and his co-workers in rabbits. They used benzene exclusively for producing leukopenia and infected their animals by intratracheal insufflation (Lamar and Meltzer). The greater part of the experiments were made with one culture of pneumococcus for the sake of uniformity. Winternitz and Hirschfelder[8] and Kline and Winternitz[9] found that pneumonia is more fatal in aplastic than in normal animals. Animals treated in like manner with toluene, a similar chemical substance causing no leukopenia, showed no decreased resistance. Animals responding to the pneumococcus infection with a leukocytosis or those having a leukocytosis produced by nutrose (sodium caseinate) seemed to have an increased resistance. In a subsequent paper Winternitz and Kline[10] find that after the intravenous injection of pneumococci in immunized rabbits the immediate reaction is not decisive of the ultimate result. The immunity process seems to be dependent on at least three factors: immune bodies, white blood cells, and a third factor which is dependent for its existence on the presence of the white blood cells at the time of the inoculation of the pneumoccoci. This third factor may be removed by rendering an immunized normal rabbit aplastic and then injecting it

[5] Ibid., 1920, 26, p. 330.
[6] Mittl. a. d. Grenzgebieten der Med. u. Chir., 1919, 19, p. 141.
[7] Jour. Exper. Med., 1915, 22, p. 174.
[8] Jour. Exper. Med., 1913, 17, p. 657.
[9] Ibid., 1913, 18, p. 50.
[10] Ibid., 1915, 21, p. 320.

with a minimal lethal dose of pneumococci. The result of the injection of this antigen into immune rabbits varies according to the presence of the three factors mentioned. The immune bodies cause an immediate disappearance of the organisms from the circulation. The third factor causes the permanent absence of the organisms from the circulation and the recovery of the animal. The white blood cells seem to be essential for the production of this third factor.

In the meantime, a reclassification of the pneumococcus into four distinct immunologic groups has been developed (Neufeld and his associates, 1910, Cole and co-workers [11]) and streptococcus hemolyticus has been divided by Avery and Cullen [12] into those from bovine sources and those from human sources, the two groups differing essentially in their ability to produce acid in dextrose mediums.

The following studies were made using the four different types of pneumococci and human and bovine strains of hemolytic streptococci. The experiments were planned to gain a general conception whether there is any difference between the effects of the roentgen ray and thorium X, both of which produce a leukopenia, on the various types of pneumococcus or streptococcus infections, and also to note whether these reagents or the leukopenia produced by them would have any effect on the invasive power or pathogenicity of the bacteria for the white mouse. Two criterions were used to determine the latter—the death of the infected and treated mice as compared to mice only infected, and the rate of appearance of the bacteria in the circulating blood.

EXPERIMENTS WITH THE ROENTGEN RAY

In all the experiments with the roentgen ray a nonlethal exposure was given the mice coincident with, or a few hours before, intraperitoneal injection. The mice, in a small box, were exposed about 6 to 12 at once for 10 minutes (Coolidge tube, the target being 8 inches from the base of the box, and using a 5 milliampere current backing up 8 inches of spark). Since it was found that the virulence of a culture of pneumococci for mice could vary in different transplants, as also noted by Wadsworth,[13] the mice were inoculated with varying amounts of the cocci in each experiment, the injections per

[11] Avery, Oswald T.; Chickering, H. T.; Cole, Rufus, and Dochez, A. R., Monographs of the Rockefeller Institute for Med. Res., 1917, 7.

[12] Jour. Exper. Med., 1919, 29, p. 215.

[13] Abst. of Bacteriol., 1920, 4, p. 20.

mouse ranging approximately from 100, 10,000, 100,000 to 100,000,000 cocci. In this way a suitable dilution was usually found that would give a consistent and distinct result in a series of mice.

TABLE 1

THE EFFECT OF ROENTGEN RAY ON PNEUMOCOCCUS AND STREPTOCOCCUS INFECTIONS IN MICE: EFFECT ON THE MORTALITY

Micro-organism		Approximate Number of Organisms Injected Intraperitoneally	Roentgenized (+) or Not (—)	Number of Mice Injected	Average Duration of Life in Hours
Name	Type				
Pneumococcus	1	100	—	4	Lived*
			+	4	88
		10,000	—	3	72
			+	4	28
		100,000	—	3	48
			+	3	30
		100,000,000	—	4	32
			+	4	22
Pneumococcus	2	100	—	3	Lived
			+	4	Lived
		10,000	—	3	78
			+	3	52
		100,000	—	4	68
			+	3	54
		100,000,000	—	3	52
			+	3	31
Pneumococcus	3	100	—	3	Lived
			+	3	Lived
		10,000	—	3	Lived
			+	4	81
		100,000	—	3	Lived
			+	3	Lived
		100,000,000	—	3	76
			+	3	52
Pneumococcus	4	10,000	—	3	Lived
			+	2	Lived
		100,000	—	3	79
			+	3	38
		100,000,000	—	2	50
			+	2	24
Streptococcus	Human	100	—	3	Lived
			+	3	60
		100,000	—	2	Lived
			+	3	61
		100,000,000	—	2	68
			+	2	42
Streptococcus	Bovine	100	—	3	Lived
			+	3	Lived
		100,000	—	2	Lived
			+	3	Lived
		100,000,000	—	2	65
			+	3	28

* The observations were made over a period of 5 days. Animals not dying before this time were recorded as living.

The summarized results of the experiments with the roentgen ray are given in tables 1 and 2 for the 6 different types of cocci.

An examination of table 1 reveals that mice subjected to a non-lethal dose of roentgen ray given shortly before infection and capable of distinctly reducing the number of peripheral leukocytes increases the pathogenicity of both pneumococci and Strept. hemolyticus, as indicated by the mortality. The pathogenicity for mice of both pneumococci and hemolytic streptococci seemed to be affected alike by the roentgen ray.

TABLE 2

THE EFFECT OF ROENTGEN RAY ON PNEUMOCOCCUS AND STREPTOCOCCUS INFECTIONS IN MICE: APPEARANCE OF COCCI IN AND DISAPPEARANCE FROM THE BLOOD

Micro-organism		Approximate Number of Organisms Injected Intraperitoneally	Roentgenized (+) or Not (—)	Number of Mice Injected	Average Time of Appearance in the Blood	Disappearance from the Blood
Name	Type					
Pneumococcus	1	100	—	3	—	—
			+	4	28′ and 34′ in 2 (2—)*	64′ and 42′ in 2
		10,000	—	3	26′ and 40′ in 2 (1—)	52′ and 50′ in 2
			+	3	Average 32′	Aver. 58′ (1 died 48′)
		100,000	—	3	Average 38′	Aver. 52′ (1 died 43′)
			+	3	Average 24′	All died 48′
		100,000,000	—	2	Average 26′	All died 50′
			+	2	Average 20′	All died 34′
Pneumococcus	2	100	—	3	46′ in 1 (2—)	46′ in 1
			+	3	36′ and 42′ in 2 (1—)	50′ and 42′ in 2
		10,000	—	3	Average 42′	Average 52′
			+	3	Average 32′	Aver. 60′ (1 died)
		100,000	—	2	Average 24′	48′ in 1 (1 died)
			+	3	Average 20′	All died 32′
Pneumococcus	3	100	—	3	—	—
			+	3	—	—
		10,000	—	2	—	—
			+	3	—	—
		100,000	—	3	32′ in 1 (2—)	40′ in 1
			+	3	Average 32′	Aver. 48′ (1 died)
Pneumococcus	4	100	—	2	—	—
			+	2	Average 30′	Average 48′
		10,000	—	2	—	—
			+	3	Average 24′	Aver. 52′ (2 died)
		100,000	—	3	Average 32′	Aver. 48′ (1 died)
			+	3	Average 22′	Aver. 48′ (2 died)
Streptococcus hemolyticus	Human	100	—	3	—	—
			+	3	32′ and 40′ (1—)	36′ and 40′
		10,000	—	3	Average 36′	Average 42′
			+	4	Average 24′	Aver. 56′ (2 died)
Streptococcus hemolyticus	Bovine	100	—	2	—	—
			+	3	—	—
		10,000	—	3	—	—
			+	4	Average 30′	Aver. 42′ (2 died)

* When all the animals in a set reveal a consistent result, the average of the total findings is given; when, however, one or two only were positive their individual results are given and those giving no findings are recorded, i e, 2— meaning two mice gave negative findings. Likewise when an animal died this is noted.

The results in table 2 indicate that mice exposed to a nonlethal but leukotoxic dose of roentgen ray increases the susceptibility of these animals to both pneumococcus and Strep. hemolyticus infections as is indicated by the earlier appearance and longer persistence of these cocci in the blood, as compared to controls receiving equal numbers of bacteria.

TABLE 3

THE EFFECT OF THORIUM X ON PNEUMOCOCCUS AND STREPTOCOCCUS INFECTIONS IN MICE: THE EFFECT ON THE MORTALITY

Micro-organism		Approximate Number of Organisms Injected Intraperitoneally	Given Thorium X (+) or Not (—)	Number of Mice Injected	Average Duration of Life in Hours
Name	Type				
Pneumococcus	1	100	—	3	Lived
			+	4	Lived
		10,000	—	3	80
			+	3	36
		100,000,000	—	2	38
			+	3	25
Pneumococcus	2	100	—	3	Lived
			+	3	Lived
		10,000	—	3	72
			+	3	42
		100,000,000	—	2	48
			+	2	20
Pneumococcus	3	100	—	3	Lived
			+	3	Lived
		10,000	—	3	Lived
			+	4	Lived
		100,000,000	—	3	70
			+	3	36
Pneumococcus	4	100	—	3	Lived
			+	3	Lived
		10,000	—	4	Lived
			+	4	Lived
		100,000,000	—	2	64
			+	3	22
Streptococcus hemolyticus	Human	100	—	3	Lived
			+	4	Lived
		10,000	—	3	Lived
			+	3	56
		100,000,000	—	3	65
			+	3	26
Streptococcus hemolyticus	Bovine	100	—	3	Lived
			+	3	86
		10,000	—	2	Lived
			+	3	84
		100,000,000	—	3	46
			+	4	28

EXPERIMENTS WITH THORIUM X

These experiments were conducted in a manner identical with that used in the roentgen-ray experiments, with the exception that the mice received a subcutaneous injection of a nonlethal leukotoxic

amount of thorium X prepared as described in a previous communication [14] and dissolved in salt solution, in place of the ray. In preliminary tests on mice 2 units of thorium X were found to produce a distinct leukopenia from an average normal peripheral leukocyte count

TABLE 4

THE EFFECT OF THORIUM X ON PNEUMOCOCCUS AND STREPTOCOCCUS INFECTIONS IN MICE: THE APPEARANCE OF THE COCCI AND THEIR DISAPPEARANCE FROM THE BLOOD

Micro-organism		Approximate Number of Organisms Injected Intraperitoneally	Given Thorium X (+) or Not (—)	Number of Mice Injected	Average Time of	
Name	Type				Appearance in the Blood	Disappearance from the Blood
Pneumococcus	1	100	—	2	—	—
			+	3	32′ in one	42′ in one
		10,000	—	3	36′ and 38′ in 2 (1—)	42′ and 46′ in 2
			+	3	Average 28′	Aver. 48′ (2 died)
		100,000,000	—	3	Average 28′	All died Aver. 52′
			+	3	Average 24′	All died Aver. 40′
Pneumococcus	2	100	—	2	—	—
			+	2	—	—
		10,000	—	3	30′ and 42′ in 2 (1—)	40′ and 42′ in 2
			+	4	Average 32′	Aver. 50′ (2 died)
		100,000,000	—	3	Average 32′	Average 46′
			+	4	Average 22′	All died 36′
Pneumococcus	3	100	—	2	—	—
			+	3	—	—
		10,000	—	2	—	—
			+	3	36′ in 1 (2—)	42′ in 1
		100,000,000	—	3	Average 38′	Aver. 46′ (1 died)
			+	4	Average 26′	Aver. 52′ (2 died)
Pneumococcus	4	100	—	3	—	—
			+	3	—	—
		10,000	—	3	—	—
			+	3	36′ and 38′ in 2 (1—)	40′ and 48′ in 2
		100,000,000	—	3	Average 30′	Aver. 52′ (2 died)
			+	4	Average 26′	Aver. 60′ (3 died)
Streptococcus hemolyticus	Human	100	—	3	—	—
			+	3	—	—
		10,000	—	2	—	—
			+	3	Average 36′	Average 40′
		100,000,000	—	3	30′ and 34′ in 2 (1—)	36′ and 40′ in 2
			+	3	Average 26′	All died 42′
Streptococcus hemolyticus	Bovine	100	—	2	—	—
			+	3	—	—
		10,000	—	2	38′ in 1 (1—)	38′ in 1
			+	3	Average 34′	Aver. 44′ (1 died)
		100,000,000	—	4	Average 28′	All died 64′
			+	4	Average 20′	All died 44′

of 13,600 per c. mm. to a minimum of 2,450 on the fifth day with complete recovery to 14,000 circulating leukocytes on the twelfth day. Five lowered the total leukocytes to about 2,100 leukocytes per c. mm. and 10 units of thorium X causing a minimum of about 1,800 leukocytes, were still nonlethal to the mature white mouse when given

[14] Corper, H. J., Amer. Rev. Tuberc., 1918, 2, p. 597.

subcutaneously. Twenty units was lethal in 7 days. The dose used in these experiments was 2 units or about $\frac{1}{10}$ of the lethal dose subcutaneously. The results of the experiments with thorium X are recorded in tables 3 and 4.

From table 3 it seems justified to conclude that thorium X shortly before infection to mice in nonlethal but leukotoxic dose capable of distinctly reducing the number of peripheral circulating leukocytes, increases the pathogenicity of pneumococci and of Strep. hemolyticus, as indicated by the mortality.

These results indicate that mice given thorium X in nonlethal but leukotoxic doses are more susceptible to both pneumococcus and Strep. hemolyticus infections, as indicated by the earlier appearance and longer persistence of these micro-organisms in the circulating blood.

SUMMARY AND CONCLUSIONS

Mice subjected to a single nonlethal exposure to the roentgen ray, capable, however, of producing a leukopenia, or given a single nonfatal injection of thorium X, also capable of causing leukopenia, and shortly thereafter inoculated with pneumococci (4 types) and hemolytic streptococci, human and bovine, revealed an increased susceptibility to all of these organisms, as is indicated by the increased and earlier mortality among the treated animals and the earlier appearance in and longer persistence of the cocci organisms in the blood, as compared with animals subjected only to inoculation.

These observations are significant since they reveal a similar increased susceptibility of the mouse subjected to these manipulations to all of the organisms tested, and bear out the results of Winternitz and his co-workers who used benzene and the pneumococcus, and Läwen, who used the roentgen ray as a leukotoxic agent and staphylococci and pyocyaneus, anthrax and typhoid bacilli as the infecting organisms. The tubercle bacillus, however, stands out distinctly from these acute micro-organisms in this respect, as noted by Corper,[2] Kellert [3] and Weinberg,[17] the course of tuberculosis in guinea-pigs being uninfluenced by the leukotoxic agents. The explanation for this difference is probably associated with the relatively greater importance of various immune processes and the defensive functions of the circulating leukocytes in the acute diseases than in chronic diseases like tuberculosis.

[17] Arch. Int. Med., 1920, 25, p. 565.